

WHAT IS CLAIMED IS:

1. A cytochrome *c* oxidase complex having cytochrome *c* oxidase activity, which complex is obtainable by the isolation from a *Gluconobacter oxydans* DSM 4025 microorganism.
2. A cytochrome *c* oxidase complex according to claim 1, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.
3. A cytochrome *c* oxidase complex according to claim 1, wherein the complex has the following properties:
 - (a) comprising at least two core subunits of I (COI) and II (COII), wherein the apparent molecular mass of COI and COII are about 43 O 10 kDa and 36 O 10 kDa, respectively by SDS-PAGE; and
 - (b) providing an absorption spectrum showing an *aa3*-type cytochrome *c* oxidase peak at 605 O 1 nm in reduced minus oxidized difference spectrum.
4. A cytochrome *c* oxidase complex according to claim 1, wherein the isolated complex is substantially homologous to a native cytochrome *c* complex from *Gluconobacter oxydans* DSM 4025 or a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.
5. A cytochrome *c* oxidase complex according to any one of claims 1-4, which is a recombinant enzyme.
6. A cytochrome *c* oxidase complex according to claim 5 comprising a core subunit containing the amino acid sequence of SEQ ID NO: 2.
7. A cytochrome *c* oxidase complex according to claim 6 comprising an amino acid sequence having 85% or greater sequence identity with SEQ ID NO: 2, and having cytochrome *c* oxidase activity.
8. A cytochrome *c* oxidase complex according to claim 5 comprising at lease one amino acid sequence selected from the group of SEQ ID NO: 4, 6 or 8.

9. A cytochrome *c* oxidase complex according to claim 8, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 4, 6 or 8, and is capable of providing the complex with cytochrome *c* oxidase activity.

10. A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

11. A recombinant polypeptide according to claim 10, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 2, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

12. A recombinant polypeptide according to claim 10, which is encoded by the polynucleotide sequence of SEQ ID NO: 1.

13. A recombinant polypeptide according to claim 12, wherein the polynucleotide sequence encodes SEQ ID NO: 2 or an amino acid sequence having at least 85% identity with SEQ ID NO: 2 and being capable of providing the complex with cytochrome *c* oxidase activity.

14. A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 4.

15. A recombinant polypeptide according to claim 14, wherein the polypeptide has an amino acid sequence that is at least 85% identical to SEQ ID NO: 4, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

16. A recombinant polypeptide according to claim 14, which is encoded by a polynucleotide sequence of SEQ ID NO: 3.

17. A recombinant polypeptide according to claim 16, wherein the polynucleotide encodes SEQ ID NO: 4 or an amino acid sequence having at least 85% identity with SEQ ID NO: 4 and being capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

18. A recombinant polypeptide comprising an amino acid sequence of SEQ ID NOs: 6 or 8.

19. A recombinant polypeptide according to claim 18, wherein the amino acid sequence is at least 85% identical to either SEQ ID NOs: 6 or 8, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

20. A recombinant polypeptide according to claim 18, which is encoded by a polynucleotide selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 7.

21. A recombinant polypeptide according to claim 20 capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity, which is encoded by a polynucleotide selected from the group consisting of a polynucleotide encoding SEQ ID NO: 6, a polynucleotides encoding SEQ ID NO: 8, a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 6, and a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 8.

22. A recombinant polynucleotide fragment comprising the polynucleotide sequence of SEQ ID NO: 1.

23. A recombinant polynucleotide fragment comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2.

24. A recombinant polynucleotide fragment according to claim 23 capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity, wherein a polynucleotide sequence encodes an amino acid sequence that is at least 85% identical to SEQ ID NO: 2.

25. A recombinant polynucleotide fragment comprising the polynucleotide sequence of SEQ ID NO: 3.

26. A recombinant polynucleotide fragment comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4.

27. A recombinant polynucleotide fragment according to claim 25 capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity, wherein a polynucleotide sequence encodes an amino acid sequence that is at least 85% identical to SEQ ID NO: 4.

28. A recombinant polynucleotide fragment comprising the polynucleotide sequence of SEQ ID NO: 5 or 7.

29. A recombinant polynucleotide fragment comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NOs: 6 or 8.

30. A recombinant polynucleotide fragment according to claim 27 capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity, wherein a polynucleotide sequence encodes a polypeptide that is at least 85% identical to SEQ ID NO: 6, or a polynucleotide that encodes a polypeptide that is at least 85% identical to SEQ ID NO: 8.

31. An expression vector comprising one or more recombinant polynucleotide fragments selected from the group consisting of recombinant polynucleotide fragments encoding SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, wherein the expression vector is suitable for expression in an organism.

32. An expression vector according to claim 31 capable of expressing at least one subunit for providing the said complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity, comprising a recombinant polynucleotide fragment selected from the group consisting of a polynucleotide encodes a polypeptide that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide encoding a polypeptide that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, a polynucleotide encoding a polypeptide that is at least 85% identical to amino acid sequence of SEQ ID NO: 6 and a polynucleotide encoding a polypeptide that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8.

33. An expression vector according to claim 31, wherein the organism is a microorganism.

34. An expression vector according to claim 33, wherein the microorganism is a bacteria.

35. An expression vector according to claim 34, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*,

Acetobacter pasteurianus, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

36. An expression vector according to claim 35, wherein wherein the bacteria is *Gluconobacter oxydans* DSM 4025.

Sub-A6
37. An expression vector according to claim 36, wherein the bacteria is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

38. A recombinant microorganism comprising the expression vector of claim 31.

39. A recombinant microorganism comprising the expression vector of claim 36.

Sub-A7
40. A recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting of a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, and a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 8.

41. A recombinant microorganism according to claim 40, comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting of a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8 to express at least one core subunit for providing the said complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

42. A recombinant microorganism according to claim 40, wherein the microorganism is a bacteria.

43. A recombinant microorganism according to claim 42, wherein the microorganism is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

44. A recombinant microorganism according to claim 43, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

45. A recombinant microorganism according to claim 44, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

46. A process for producing a cytochrome *c* oxidase complex as set forth in any one of claims 1 - 9 comprising:

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(a) cultivating in a culture medium a recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 8, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8, for providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity; and

(b) recovering cytochrome *c* oxidase from the culture.

47. A process according to claim 46, wherein the recombinant microorganism is a bacteria.

48. A process according to claim 47, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

49. A process according to claim 48, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

50. A process according to claim 49, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

51. A process for producing 2-keto-L-gluconic acid (2-KGA) from L-sorbose or D-sorbitol comprising:

(a) cultivating in a culture medium a recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, and a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 8, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8 and capable of expressing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity; and

(b) recovering 2-KGA from the culture medium.

52. A process according to claim 51, wherein the recombinant microorganism is a bacteria.

53. A process according to claim 52, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

54. A process according to claim 53, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

55. A process according to claim 54, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

56. A cytochrome c oxidase complex comprising a core subunit containing a polypeptide sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8, fragments of SEQ ID NO:2 capable of providing the said complex with cytochrome c oxidase activity, and a polynucleotide sequence that encodes a polypeptide that is capable of providing the complex with cytochrome c oxidase activity, and which polynucleotide hybridizes under high stringency hybridization and wash conditions to a polynucleotide sequence encoding SEQ ID NO:2, 4, 6 or 8.

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